

Microbiological Quality and in Use Preservative Capacity of Shampoo Preparations Manufactured in Jordan

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Abstract

The microbiological quality of 16 different shampoo formulations (manufactured by 16 different factories) and marketed in Jordan was investigated to determine the preservative capacity of these products at the time of sale and after use. Procedures used were according to those described in ISO technical standards. Thirteen (81.25 %) of the formulations studied were found to be free of contamination. One product harbored *Escherichia coli* and 2 contained *Pseudomonas aeruginosa*. Total microbial count in the contaminated brands was $>10^5$ CFU / ml. When the contamination free products were returned after a period of normal use, 7 (53.85 %) harbored variety of gram negative and positive bacteria; high numbers of bacteria were detected in 5 (38.5 %) of the returned products. Cocamide diethanolamide in mineral salts medium supported the growth of *P. aeruginosa* ATCC 9027, whereas sodium lauryl ether sulphate was not inhibitory at the concentration usually used in shampoo formulations. It is concluded that unless adequately preserved, shampoo ingredients can support the growth of microorganisms known to cause spoilage and / or possible health problems. Manufacturers of shampoos in Jordan need to improve the in use preservation efficacy of their products before gaining the confidence of consumers.

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1. Introduction

Shampoo preparations are personal care products. The bulk ingredient in these formulations is water, typically making about 70 – 80% of the entire formula. The second major constituent is the primary surfactant, followed by the foam booster. Other ingredients such as thickeners, conditioning agents, modifiers and special additives are incorporated to provide the product with additional required properties (Hössel *et al.*, 2000).

However, the sterility of shampoos is not necessary; they must not be contaminated with pathogenic microorganisms and they should not harbor microbial contaminants in high numbers (Ravita *et al.*, 2009). It has long been demonstrated (Olson, 1967) that proliferation of *Pseudomonas* species in a shampoo preparation based on sodium lauryl sulphate as a surfactant resulted in product separation and discoloration. The rate of microbial contamination in shampoo brands marketed in a developing country was found to be 43% (Abdelaziz *et al.*, 1989). These authors found that bacterial count in the investigated products was low and pathogens were absent. Of special concern to cosmetic industries is the detection of *Pseudomonas aeruginosa* in their products. This bacterium is an opportunistic pathogen with spoilage potentials and was the most common microorganism

associated with recall of cosmetic formulations in the United States and Europe (Wong *et al.*, 2000; Lundov and Zachariae, 2008)

Assessment of preservative efficacy in cosmetics is usually performed using the challenge test (ISO/WD 11930: 2008). This test provides assurance regarding the microbiological quality of the product at the time the test is performed. Russell (2003) suggested that challenge test should be undertaken at the beginning, during and at the end of the shelf life of the product.

Jordan institution for standards and metrology adopted a hair shampoo specification (JS 483: 2002) which has become mandatory since January 2003. This specification stipulates that total bacterial count should be $< 10^2$ / ml of shampoo and gram negative bacteria as well as *Staphylococcus aureus* should be absent. Unfortunately, there is no published work from Jordan that deals with this subject; the objective of this paper is to investigate the microbiological quality of shampoo brands manufactured and marketed in Jordan prior and during normal use by consumers. The ability of *P. aeruginosa* to grow at the expense of sodium lauryl ether sulphate and cocamide diethanolamide, which are the major two surfactants used in shampoo formulations was also studied.

2. Materials and Methods

A total of 16 different shampoo brands were purchased from the local market with preference to preparations

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manufactured by Jordanian companies. Each brand was manufactured by a company and was given a code in the laboratory before the experimental work was commenced. Viable bacterial count was performed for each product using the pour plate technique. Aliquot of 1.0 ml of the respective preparation was aseptically transferred to a sterile petridish and 20 ml of molten tryptic soya agar supplemented with 1% tween 80 as a neutralizing agent. The plate was shaken, allowed to solidify and then incubated at 35 °C for 48 hours before developed colonies were counted visually. When initial colony count was high, 10 fold serial dilutions were made in sterile saline and pour plate was repeated. Developed colonies were purified and identified according to the diagnostic tables given by Barrow and Feltham (1993). The entire identification tests employed were all traditional based on gram reaction, microscopy, biochemical reactions and ability to grow on certain substrates. Yeast and moulds were recovered by directly plating a loop-full of the preparation onto the surface of a dried Sabouraud Dextrose Agar, which were then incubated at room temperature (approximate to 22- 25 °C) and inspected daily for the presence of growth before being discarded after 7 days. Isolation of *E.coli*, *P.aeruginosa* and *Staphylococcus aureus* was performed as described in ISO technical publications (22717: 2006, 22718: 2006 and 21150: 2006, respectively).

To determine the in-use efficacy of the preservative system in the shampoo brands that were found to be free of microbial contaminants, formulations were given to volunteers for normal use and then returned when the residual quantity in the container was approximate to 1/4 of its original volume (no time limit for use was given to volunteers as the container volume varied from one brand to another; but in general they were all returned in less than a month). Microbial count in the remaining aliquots of the used shampoo and the identity of the bacterial isolates in addition to the detection of specific microorganisms were all performed as given above.

The ability of *P. aeruginosa* ATCC 9027 to survive and grow at the expense of sodium lauryl ether sulphate (SLES), which is a major surfactant, used in shampoo formulations and cocamide diethanolamide (CDEA), which is a foam booster, was established using mineral salts medium supplemented with either of the compounds as a main source of carbon and energy. Increase or decrease in number of the inoculated bacterial strain employed in this experiment was plotted in a growth curve and the assimilability of the material under investigation by the test organism was extrapolated. A cell suspension of *P. aeruginosa* ATCC 9027 was prepared by growing the bacterium for 24 hours on a plate of nutrient agar; colonies were harvested and then suspended in phosphate buffer to contain 10^7 CFU / ml. Aliquot of 1 ml of this suspension was used to inoculated 100 ml of sterile Bushnell - Has broth medium (this is a mineral salts medium devoid of any carbon source) in 500 ml flasks supplemented with either 14.5 gram of commercial SLES or 4 g of CDEA. Flasks were incubated in a shaking water bath and at intervals 1 ml aliquots were aseptically withdrawn for total viable bacterial count as given above. A flask containing Bushnell - Has broth without any carbon source was also inoculated with the same bacterial suspension to serve as a

control. This was incubated and its content sampled as the other 2 flasks. All media used throughout this work were purchased from Difco -Michigan-USA

3. Results

Thirteen out of 16 shampoo preparations manufactured and marketed in Jordan were found to be free of microbial contamination. Selected organisms, namely *Pseudomonas* species, *E. coli*, *S. aureus*, *Candida* species and moulds were not detected. The remaining 3 brands were found to be heavily contaminated; total bacterial count in the 3 products exceeded 10^5 CFU / ml. One product harbored *E. coli* while the other 2 were contaminated with *P. aeruginosa*.

The formulations which were found of acceptable quality were given to volunteers for normal use. When approximately 3/4 of the bottle content was used, they were returned for further processing. Seven out of 13 returned products harbored microorganisms to various levels, varying from 70 to 10^5 CFU/ ml. Table 1 demonstrates types and numbers of bacteria recovered from shampoo products after being used. In brief, 2 products sustained count < 100 CFU / ml whereas, the other 5 contained high numbers (> 10^2 CFU / ml). *Bacillus* species was the most dominant bacteria; whereas each of the following bacteria; *Pseudomonas* sp., coagulase negative Staphylococci and *Enterobacter* sp. were recovered from 3 products. *Serratia* sp was detected in 2 of the returned products. However, *E. coli*, *Staphylococcus aureus*, *Candida* and moulds were not isolated.

Figure 1 demonstrates the growth curves of *P. aeruginosa* ATCC 9027 in Bushnell-Has medium with and without SLES or CDEA. It is evident from this figure that commercial grade of SLES cannot be considered as inhibitory to the test organism at the concentration used (14.5 g %). On the other hand, CDEA was definitely nutritional to the test organism at the concentration employed in the experiment.

4. Discussion

This investigation has demonstrated that 18.75 % of the studied shampoo products were heavily contaminated with bacteria (> 10^5 CFU/ ml). Contaminants included *P. aeruginosa* and *E. coli*. The former organism is an opportunistic pathogen with spoilage potential and the later is an indicator of fecal pollution. According to the Jordanian standards (JS 483: 2002) shampoo formulations should not contain more than 10^2 CFU / ml and *P. aeruginosa* as well as *E. coli* must be absent. Therefore these brands were out of specifications and consequently they were not included in the in-use preservative efficacy studies.

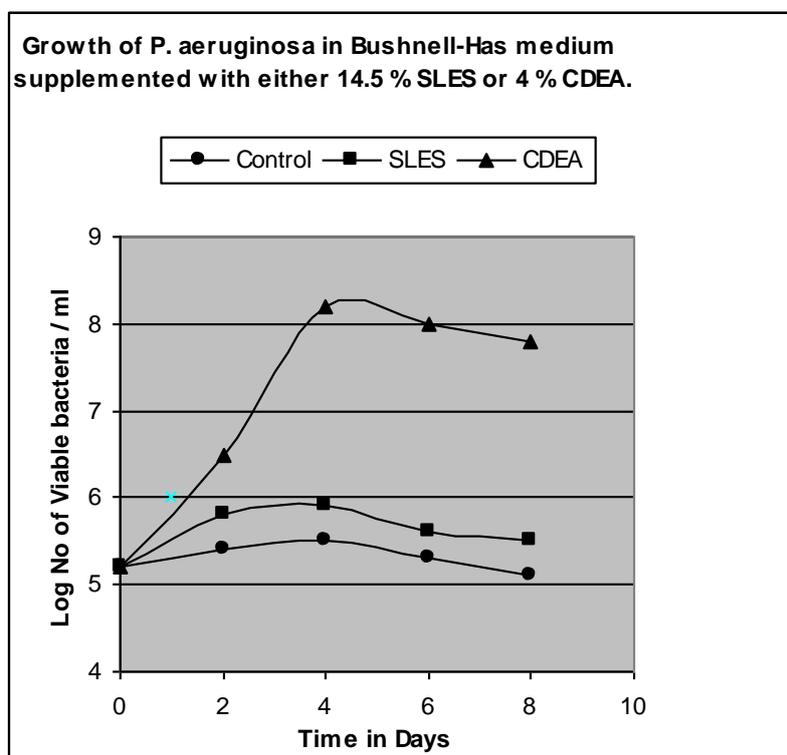
Abdelaziz *et. al* (1989) studied 8 commercial brands of shampoo in Egypt and found that none of the formulations they studied harbored microorganisms in excess of 10^4 CFU / ml. Only 15 % of the products revealed bacterial count between 102 to 103 cells / ml. These observations are very close to ours as 15% of their investigated formulations can be considered as out of specifications according to the Jordanian standards.

Table 1. Types and Numbers of Bacteria Isolated from Shampoo Products after Normal Use.

Product code	CFU/ ml	Type of bacteria isolated
B	3×10^2	<i>Bacillus</i> sp. and <i>Serratia</i> sp.
D	70	<i>Bacillus</i> sp, <i>Staphylococcus</i> sp*
E	4×10^5	<i>Bacillus</i> sp, <i>Pseudomonas</i> sp, and <i>Enterobacter</i> sp
H	81	<i>Bacillus</i> sp and <i>Staphylococcus</i> sp*
J	5×10^2	<i>Bacillus</i> sp, <i>Enterobacter</i> sp. and <i>Pseudomonas</i> sp
K	2×10^4	<i>Bacillus</i> sp, <i>Serratia</i> sp. and <i>Pseudomonas</i> sp.
M	4×10^3	<i>Bacillus</i> sp, <i>Staphylococcus</i> sp* and <i>Enterobacter</i>

* Coagulase negative Staphylococci

Figure 1. Growth Pattern of *Pseudomonas aeruginosa* ATCC 9027 in Mineral Salts Medium (Busnell-Hass) Supplemented with either 14.5 % SLES or 4 % CDEA



In comparison, 18.75% of the shampoo products investigated in this work was found to be out of microbiological limits. These results are not surprising as microbial contamination of current cosmetics particularly

shampoos were encountered in various countries (Campana *et al.*, 2006; Lundov *et al.*, 2008).

Unless they are properly preserved, cosmetics provide microorganisms with adequate environments for their

growth. Preservatives are incorporated into formulations to maintain the microbial load in these products to a safe and acceptable level. In order to establish the preservative efficacy of a given formulation, a challenge test is performed (ISO/WD 11930: 2008). This test provides information's regarding the capacity of the preservative system to cope with the actual challenge to which the formulation is usually exposed during use (Russell 2003; Campana, *et al.*, 2006). Another inference from the challenge test is to allow volunteers to use the product for a certain period of time and then test the product for the presence of microorganism (Brannan and Dille, 1990). This is exactly the approach which was adopted in this investigation.

Table 1 shows that after normal use 7 (53.9%) out of the 13 brands harbored viable microorganisms, 2 (15.4%) were within the acceptable limit ($<10^2$ CFU / ml) and 5 (38.5%) were out of limit. These findings cannot be compared with any other work as there is no published literature directly related to post use microbiological quality of commercial shampoo brands. The closest to this work was that performed by Brannan and Dille (1990) who investigated a prototype shampoo formulation, containing no preservatives. These authors established that dispensing closure used for shampoo containers played an important role in protecting cosmetics from in-use microbial contamination. However, detection of *Pseudomonas* sp. and *Serratia* sp. in the returned post use shampoos is consistent with those reported by Brannan and Dille (1990), the difference being in the recovery of *Bacillus* sp. and coagulase negative Staphylococci; these organisms were present in our study but absent in theirs. Nevertheless, the later two bacterial types were isolated from commercial brands marketed in Egypt (Abdelaziz *et al.*, 1989).

The isolation of a variety of gram negative and positive bacteria from 53.85% of post use shampoos raise the obvious question of why preservatives in these products failed to deal with consumers challenges although they were definitely effective when containers were just opened. It is feared that the majority of these products was preserved with formaldehyde (formalin) which has the tendency to evaporate when the container is opened and thus leaving the product without preservation. However, formaldehyde in low concentration is still used for the preservation of cosmetics; high amounts of this compound could be extremely toxic (Rivero and Topiwala, 2004; Yazar *et al.*, 1010; Lundov *et al.*, 2010). The frequency of use and concentration of formaldehyde in cosmetics manufactured in Jordan is worth a comprehensive investigation.

It is assumed that microbial growth in cosmetics is contingent on the ability of the contaminant to utilize product formulation as carbon and energy source. The range of chemicals used in shampoo formulations is so versatile and thus contaminants will always find chemically needed growth requirements in this man made habitat. SLES is added to shampoos as a primary surfactant while CDEA is employed as a foam booster and both are used in large amounts. It has long been argued that anionic surfactants such as SLES may exhibit antimicrobial properties but unfortunately this argument

has not been supported by experimental data (Bryce and Smart, 1965).

It is evident from Figure 1 that SLES at a concentration of 14.5% in mineral salts medium was not inhibitory to *P. aeruginosa* ATCC 9027. In the contrary, almost 7 fold increases in the initial number of the test organism was observed after 2 days of incubation as compared to the viable cells detected in the control medium which was devoid of any carbon source. It is important to note that the concentration of active matter in the commercial grade of SLES used was 70%. Therefore the slight growth obtained could be attributed to the available impurities within the compound and not to the surface active agent itself. On the other hand, *P. aeruginosa* ATCC 9027 was very prolific in mineral salts medium supplemented with 4% CDEA as amain source of carbon and energy. Total amide content in the commercial CDEA was a minimum of 96%.

In this context, it is worthwhile to refer to the statement given by Scott and Gorman (1992) which says that anionic and non-ionic surface active agents have strong detergent properties but exhibit little or no antimicrobial activity. This paper has clearly shown that while CDEA was readily utilizable by Pseudomonad, SLES showed no inhibitory effect against the same organisms.

In conclusion, shampoo preparations based on SLES and CDEA provide microorganisms with environment conducive for their growth and will remain susceptible to microbial attack during use. Manufacturers of these products should use adequate preservative systems, capable of dealing with contaminants that are likely to gain entrance into the product during the production process or normal use by consumers. Cosmetic companies in Jordan should pay special attention to this problem before gaining the confidence of the public.

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